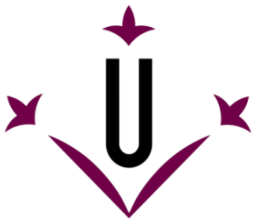

Vitamin-D pathway genes and HIV-1 disease progression in injection drug users

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Abstract

Vitamin-D has pleiotropic effects on calcium and bone metabolism, cellular growth control, cell differentiation and modulation of both innate and acquired immune response. Previous studies revealed the association of vitamin-D receptor gene (VDR) polymorphism with infection diseases including HIV-1 infection. To assess for association between polymorphisms of vitamin-D pathway genes CYP27B1, vitamin-D binding protein (VDBP) and VDR with HIV-1 infection, disease progression to acquired immunodeficiency syndrome (AIDS) was analysed according to CDC93 criteria in a cohort of 185 HIV-1 seroprevalent patients belonging to the injection drug users. Genotype data was obtained from rs10877012, rs3782130 and rs4646536 markers at CYP27B1 locus; rs7041 and rs4588 at VDBP locus; and rs11568820, rs4516035, rs2228570, rs1544410 and rs17878969 at VDR locus. Distribution of genotypes between patients grouped by outcome was compared by contingency table analysis. Marker–marker interaction was assessed by a MDR analysis. Assuming an additive model for VDR markers, a Kaplan–Meier survival analysis was employed to evaluate association with disease progression. Among vitamin-D pathway genes, VDR locus reveals specific 5′UTR and 3′UTR diplotype combinations associated with both, slower and faster progression to AIDS. Marker–marker interaction analysis indicates a strong interaction between VDR markers and a redundant effect for CYP27B1 markers. According to our results, VDR locus association follows an additive model in which increased genetic risk score for the VDR is directly correlated with AIDS progression rates. Our data supports a role of vitamin-D pathway gene variability on HIV-1 disease Progression

1. Introduction

In addition to its role on mineral metabolism, 1α,25-dihydroxyvitamin-D3 (henceforth vitamin-D) has pleiotropic effects on cellular growth control, cell differentiation and modulation of the immune response. A growing body of experimental evidence has been obtained in the last decade that supports a key role of vitamin-D in the control of both innate and acquired immune responses (Adams, 2006; Hewison, 1992, 2008; White, 2008). Following the activation of vitamin-D precursors in the skin by sunlight exposure and their biochemical transformation in the liver, vitamin-D acquires its full active form after being converted to vitamin-D by the kidney enzyme 25-hydroxyvitamin-D3 1-α-hydroxylase (CYP27B1) (Lips, 2006). Under normal physiological conditions, nearly all circulating vitamin-D compounds are bound to the vitamin-D-binding protein (VDBP) that transports vitamin-D metabolites to the target tissues (Daiger et al., 1975). At the molecular level vitamin-D excerpt its action by interacting with the nuclear vitamin-D receptor (VDR), that acts as a transcription factor activating or repressing specific genes (Pike, 1991).

Vitamin-D pathway genes have been largely evaluated in disease by their implication in the immune response regulation. CYP27B1 and VDR are expressed in several immune cells, such as macrophages, dendritic cells (DCs) and lymphocytes. Identification of extra-renal sites for CYP27B1 and VDR expression has led to hypothesize that local production of vitamin-D could play an important autocrine or paracrine role in the differentiation and function of these cells (Bouillon et al., 1995a, 1995b; Haussler et al., 1998; Walters, 1992). Macrophages and DCs, as they differentiate towards a mature antigen-presenting phenotype, show simultaneously an increase on CYP27B1 expression

and a decrease on VDR expression (Hewison et al., 2003). As a consequence, immature DCs are able to respond to vitamin-D, suppressing their differentiation by a negative feedback control (Hewison et al., 2004). Moreover, in addition to its role on vitamin-D transport, vitamin-D-binding protein derived Macrophage Activating Factor (GcMAF), is also involved in the immune response acting as a chemotactic factor in the recruitment of neutrophil leucocytes (Kew and Webster, 1988).

A potential role of vitamin-D on human immunodeficiency virus Type 1 (HIV-1) infection has been previously considered (Villamor, 2006; Vincek, 1995) and comprehensively reviewed in Fibla and Caruz (2010). Two processes are crucial against HIV-1 infection in which vitamin-D plays opposite effects, the synthesis of antimicrobial peptides and the role of T-helper mediated response. On one hand the hormone promotes the synthesis of peptides that exert antimicrobial effect to the virus entry (Quiñones-Mateu et al., 2003), whereas on the other hand, induces the polarization of the immune response towards a less effective T-helper response against viral infections. Noteworthy, vitamin-D is known to alter both, Th1/Th2 and Th17/Treg balances, to Th2 and Treg responses, that in the context of viral infections should be considered detrimental (Bruce et al., 2010).

Almost normal levels of circulating vitamin-D have been described in HIV-1 infected patients without acquired immunodeficiency syndrome (AIDS) events. In contrast these levels were strongly reduced during disease progression and directly correlated with survival (Haug et al., 1994). Although vitamin-D deficiency in AIDS progression has not been related to 1α-hydroxylase dysfunction, it has been described that protease inhibitors used in the treatment of HIV-1 infected patients interfere with vitamin-D metabolism by

inhibiting 25-hydroxylase, 24-hydroxylase and 1α-hydroxylase activities (Cozzolino et al., 2003).

Association of VDR gene polymorphisms have been reported with disease progression rates in patients infected by HIV-1 (Barber et al., 2001; Moodley et al., 2013; Nieto et al., 2004). Several studies revealed the association between CYP27B1 polymorphisms and immune related diseases, such as Hashimoto's thyroiditis, Graves' disease and type 1/2 diabetes (Lopez et al., 2004) and Addison's disease (Jennings et al., 2005). VDBP polymorphisms have been associated with HIV-1 infection (Eales et al., 1987) but these findings have not been replicated (Alonso et al., 1990; Cleve et al., 1988). In addition, VDBP variants have been associated with susceptibility to tuberculosis infection (Hewison, 2008; Martineau et al., 2010; White, 2008) and asthma (Li et al., 2011; Lips, 2006) and VDBP has been proposed as a multiple sclerosis biomarker (Disanto et al., 2010).

In the present study we have evaluated vitamin-D pathway genes CYP27B1, VDBP and VDR as candidate genes involved in HIV-1 disease progression.

2. Materials and methods

2.1. Study participants

The main characteristics of the HIV-1 infected cohort have been previously described (Barber et al., 2001; Nieto et al., 2004). Briefly, the Lleida AIDS Cohort is a prospective seroprevalent cohort of HIV-1 infected patients belonging to the intravenous drug users risk group drawn from all HIV-1 seropositive adults enrolled in the AIDS Service of the

University Hospital Arnau de Vilanova. Only Caucasian patients recruited between 1982 and 1991 were included in the cohort. All patients selected were in follow-up for more than 7 years (median, 127.7 months; range 84–198 months). Progression criteria have been established according to the 1993 Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS Among Adolescents and Adults of the Center for Disease Control classification (CDC93) that considers both clinical and immunological parameters (Anonymous, 1993). In addition to AIDS-defining illness considered in the 1983-CDC AIDS-defining criteria, the 1993-CDC AIDS-defining criteria expanded the AIDS surveillance case definition to include all HIV-infected people with CD4+ T-lymphocyte counts of less than 200 cells/μL. Epidemiological and clinical characteristics of the HIV-1 seropositive patients have been presented in Barber et al. (2001), Nieto et al. (2004) and Laplana et al. (2013) and are summarized according to CDC93 progression status in Supplementary Table 1. The observation period for progression status ended in December 1999. All participants gave written informed consent. The Ethics committee from our institution approved the study.

2.2. DNA sources and genotyping

Genomic DNA was extracted from peripheral blood lymphocytes using a KG-Midi extraction kit (Camgen, Cambridge, UK). Selected single nucleotide polymorphisms (SNP) were; rs4646536, rs3782130 and rs10877012 from the CYP27B1 gene; rs7041 (Glu416Asp) and rs4588 (Thr420Lys) from VDBP gene and rs17878969 (PolyA), rs1544410 (Bsm-I), rs2228570 (Fok-I), rs4516035 (A1012G) and rs11568820 (Cdx) from the VDR gene. Polymerase Chain Reaction (PCR) protocols were developed to genotype the 10 SNP, the main characteristics of the genotyping methods are described

in Supplementary Table 2. For each SNP, assay validation was performed using representative individuals whose genotypes were previously determined by sequencing.

2.3. Statistical analysis

We examined the relationship between single SNP at CYP27B1, VDBP and VDR loci with AIDS disease status by conducting a cohort-based study. Hardy–Weinberg equilibrium was tested comparing expected and observed genotype frequencies by Chi-square test. We used Haploview 4.2 software (Barrett et al., 2005) to estimate linkage disequilibrium among the analysed markers and pLink software (Purcell et al., 2007) to infer haplotype and diplotype frequencies fromour sample data.

Allele, genotype, haplotype and diplotype frequencies were compared among patients categorized as progressors and non-progressors according to the CDC93 that considers both clinical and immunological parameters. Single-marker association P-values were corrected for multiple testing following the SNPspectral decomposition approach, a modified Bonferroni-corrected nominal threshold of P= 0.05/N, where N is the "effective number of independent marker loci" after consideration of linkage disequilibrium between markers. N was calculated using the web-based program SNPSpD (http://gump.qimr.edu.au/general/daleN/SNPSpD/). Following this, the experiment-wide significance threshold required to keep Type I error rate at 5% is P b 0.0056. Differences among genotypes in median values for numerical variables were tested by non-parametric Kruskal–Wallis test.

In addition, we studied HIV-1 disease progression rates by a Kaplan–Meier survival analysis in patients stratified according to genotype/diplotype groups. Differences between groups were tested by Log-Rank test. Hazard ratios were estimated using a Cox proportional hazard model (HRu) and adjusting for sex, age at first HIV positive test and CCR5Δ32 genotype (as previously reported for this cohort in Barber et al. (2001) (HRa). Survival time ranges from date of the first HIV-1 positive test to outcome or censoring date (last clinic examination date or date of death if not caused by HIV-1 infection). Five patients died after reaching the outcome and 1 patient died before reaching outcome by heroine overdose. This patient was assumed as outcome free as remained more than seven years with CD4 N 200/μL and free of antiretroviral therapy. A $P = 0.05$ was considered as statistically significant. Statistical analyses were performed by the SPSS 20.0 package.

2.4. Interactive effects between markers on HIV-1 progression by MDR Analysis

The interactive effect between CYP27B1, VDBP and VDR genes was assessed by multifactor dimensionality reduction (MDR) using MDR software (Hahn et al., 2003). Multifactor dimensionality reduction is a non-parametric data mining approach, which pools multi-locus genotype/diplotype with high dimensions into one dimension model. The MDR approach was employed to enumerate all possible combinations of the considered loci, with various model lengths. MDR evaluates the predictor using cross-validation method and permutation testing. The combinatorial examination by these two approaches would minimize false positive rates. Cross-validation consistency (CVC) and prediction accuracy were calculated for each combination of tested genotypes/diplotypes. The highest CVC value, which indicates the number of times that a particular set of factors is identified in each possible 9/10 combination of the patients, and the highest

prediction accuracy value, an average of accuracies calculated on all the validation datasets, are pointing out the best model. The Sign test gives a P-value for each best model adjusted for multiple testing.

3. Results

3.1. Allele and genotype distribution

Markers analysed show no departure from Hardy–Weinberg equilibrium. We compared genotype and allele frequencies in patients classified as progressors (N=89) and non-progressors (N=96) according to CDC93. We didn't observe differences in CYP27B1 and VDBP allelic and genotypic distribution among groups. In contrast, we found statistical differences in the distribution of 5′ UTR rs4516035 (A1012G), exon 2 rs2228570 (Fok-I) and3′UTR rs17878969 (PolyA) polymorphisms at the VDR locus. Overall genotype distribution at 5′UTR rs4516035 polymorphism showed marginal differences (P= 0.027). Protection of progression to AIDS was assigned to rs4516035-G/G homozygotes based on their underrepresentation in progressors (Odds Ratio (OR) = 0.41; 95% Confidence Interval (CI): 0.19–0.89; P = 0.018). On the other hand, risk of progression to AIDS was assigned to exon 2 rs2228570 (Fok-I) heterozygotes and 3′UTR rs17878969-S/S homozygotes based on their overrepresentation in progressors (OR = 1.53; 95% CI: 1–2.3; P = 0.043 and OR = 2.2; 95%CI: 1.1–4.4; P = 0.022, respectively) (Table 1).

3.2. Haplotype and diplotype analyses

Linkage disequilibrium (LD) pattern in the sample was in accordance with available data for Caucasian populations (Fang et al., 2005) and block structure was observed at VDR locus for 5′UTR markers rs11568820:rs4516035 (D′ = 0.73) and 3′UTR markers rs1544410:rs17878969 (D′ = 0.89). In addition, markers at CYP27B1 and VDBP loci showed strong linkage disequilibrium(D′ N 0.92 and D′=1, respectively). According to the observed LD pattern, haplotypes were inferred for 5′ and 3′UTR markers at VDR locus and for all markers at CYP27B1 and VDBP loci.

Global 5′UTR VDR, 3′UTR VDR, VDBP and CYP27B1 haplotype association with progression was not statistically significant. Diplotype distribution of CYP27B1, VDBP, and 5′UTR and 3′UTR regions of VDR loci are presented in Fig. 1. No differences were found for CYP27B1 and VDBP diplotype distribution. In contrast, diplotype distributions at VDR 5′UTR showed that homozygotes for rs11568820-G:rs4516035-G (GG) haplotype were underrepresented in those individuals reaching the outcome (12.4% vs. 26%; OR = 0.4; 95%CI: 0.18–0.8; P = 0.019) while rs11568820-G:rs4516035-A_rs11568820-G:rs4516035-G (GA/GG) diplotype heterozygotes were overrepresented (36% vs. 21.9%; OR = 2; 95%CI: 1.05–3.8; P = 0.036) (Fig. 1B). In addition, noncarriers of rs1544410-G:rs17878969-L (GL) haplotype at 3′UTR VDR locus were overrepresented in those reaching the outcome (40.3% vs. 25%; OR = 1.96; 95%CI: 1.04–3.7; P = 0.034) (Fig. 1D).

3.3. Marker–marker interaction analysis

In order to evaluate the possible marker–marker interaction between CYP27B1, VDBP and VDR polymorphic variants on HIV-1 progression rates, we performed anMDR

analysis considering five independent genotype/diplotype combinations. Results are summarized in Table 2 were the best models for each number of loci combination are presented. VDR Fok-I polymorphism was found to be the strongest single-factor (CVC = 4/10, prediction accuracy = 0.5838) while the five-loci model was regarded as the optimal model (CVC = 10/10, prediction accuracy = 0.7405). Nevertheless, if we consider the Sign test P-value, we should contemplate the three-loci (VDR 5′UTR; VDR Fok-I and VDR 3′ UTR) model as the best interaction model. Supplementary Fig. 1 shows the dendrogram plot for the interactions detected by MDR analysis between all genotype/diplotype combinations. Results showed the lack of interaction between VDBP locus diplotypes and any other loci. In contrast, synergistic interaction was observed between VDR Fok-I genotypes and VDR 3′UTR diplotypes. Redundant interaction between VDR 5′UTR and CYP27B1 diplotypes and a weak interaction between the two interacting groups were found.

3.4. Disease progression analysis according to genotypes/diplotypes

Kaplan–Meier survival analysis for progression to AIDS evaluated in the entire cohort of HIV-1 infected patients showed a global mean time to progression of 133 months (95%CI: 124–142).Mean time to progression of patients does not showed statistical differences when grouped according to both, VDBP and CYP27B1 diplotypes. Considering the potential effect of treatment on CYP27B1 activity we evaluated Kaplan–Meier survival curves stratifying according to initiation of antiretroviral treatment. Our results showed that CYP27B1 variability seems to have a slight effect on progression only in not treated patients (data not shown). In contrast, mean time to progression in patients grouped according to VDR genotype/diplotypes showed significant differences. Thus, a

lower mean time to progression was observed for patients harbouring 5′UTR GA/GG diplotype (110 months, 95%CI: 97–123), Fok-I C/T heterozygotes (120 months, 95%CI: 107–133) and noncarriers of 3′UTR GL haplotype (119 months, 95%CI: 103–135). In addition, patients harbouring 5′UTR GG/GG diplotype, showed a higher mean time to progression than the observed in the entire cohort (148 months, 95%CI: 133–163).

3.5. Disease progression rates according to VDR cumulative genetic score

Based on the genotype/diplotype association results, interaction analyses and Kaplan–Meier survival analysis,we have assessed an additive unweighted model for the three-loci VDR genotype/diplotype combinations effect on HIV-1 progression rates (Salgado et al., 2011). For that purpose, we have assigned a protective score (−1) to 5′UTR GG/GG diplotype; a risk score (+1) to 5′UTR diplotype GA/GG, Fok-I C/T heterozygotes and noncarriers of 3′UTR GL haplotype; and neutral score (0) to all other genotype/diplotype combination. Assuming this model we have computed for each individual a cumulative genetic score (CGS) at the VDR locus obtained by the sum of the previously assigned scores. The computations of the VDR cumulative genetic score allow us to classify patients into five CGS groups ranging from CGS −1 to CGS+3.

Progressors were underrepresented in CGS −1 (OR = 0.23; 95%CI: 0.07–0.74; P = 0.008) and CGS 0 groups (OR = 0.43; 95%CI: 0.22–0.83; P = 0.011) while overrepresented in the CGS +2 group (OR =2.13; 95%CI: 1.1–4.2; P = 0.031) (Fig. 2A). Noteworthy, the relative prevalence of progressors grows as the cumulative genetic score increases (Fig. 2B).

Fig. 3 shows Kaplan–Meier survival curves for progression to AIDS of patients grouped according to VDR CGS groups. Mean time to outcome was significantly higher for CGS −1 group (159 months; 95%CI: 141– 177) when compared with CGS +1 (118 months; 95%CI: 105–132), CGS +2 (118 months; 95%CI: 101–134) and CGS +3 (82 months; 95%CI: 63–103) groups (Log-Rank test P = 0.004, P = 0.001 and P b 0.001, respectively). In addition, Cox proportional hazard ratio (HR) for progression of CGS −1 group was noticeably protective when comparing with CGS +1, CGS +2 and CGS +3 groups (HRu = 0.25; 95%CI: 0.09–0.7; P = 0.011; HRu = 0.22; 95%CI: 0.07–0.62; P = 0.004 and HRu = 0.11; 95%CI: 0.03–0.37; P = 0.0004, respectively), that remains significant after adjusting for sex, age at first HIV positive test and CCR5Δ32 genotype (HRa = 0.26; 95%CI: 0.09–0.77; P = 0.013; HRa = 0.21; 95%CI: 0.07–0.62; P = 0.005 and HRa = 0.11; 95%CI: 0.03–0.38; P = 0.0005, respectively).

Themain characteristics of the studied cohort arranged according to the VDR CGS groups are presented in Supplementary Table 3. Overall distribution of values showed statistical significant differences in follow-up time (P = 0.008), with shorter time in patients with higher CGS; patients distributed by CD4 cell count stages (P = 0.002), with a larger proportion of stage 3 patients in CGS groups N +1; CD4 cell count at first determination (P = 0.01) and lowest CD4 cell count (P = 0.004), with decreasing values as increased CGS value; and highest virus load (P = 0.026), with greater values in patient with higher CGS value.

4. Discussion

Based on previous works connecting VDR variability with susceptibility to HIV-1 infection (Alagarasu et al., 2009; de la Torre et al., 2008) and disease progression rates to AIDS (Barber et al., 2001; Nieto et al., 2004), we have extended the association study to additional candidate genes involved on vitamin-D physiology such as CYP27B1 and VDBP genes, as well as polymorphisms covering the entire VDR locus.

Following a step-by-step strategy, we first asked for single SNP association. Secondly, we requested for haplotype and diplotype association and interactions. Finally, after assigning unweighted genetic scores for selected genotypes/diplotypes at the VDR locus, we computed a cumulative genetic score in order to test an additive model for the association with progression to AIDS.

Single SNP analysis and haplotype/diplotype association test for selected candidate genes involved on vitamin-D physiology fail to show association with progression for markers at CYP27B1 and VDBP loci. In contrast, significant association with progression was detected for markers at VDR locus. In addition to results indicating association between rs2228570-C/T (Nieto et al., 2004), and rs1544410-A/A (Barber et al., 2001; Moodley et al., 2013; Nieto et al., 2004) genotypes with rapid progression to AIDS diplotype analysis at VDR locus reveal specific 5′UTR and 3′UTR diplotype combinations associated with both, slower and faster progression to AIDS. According to our results, VDR locus association follows an additive model in which increased genetic score for the VDR is directly correlated with AIDS progression rates. MDR analysis indicates a strong interaction between VDR markers and a redundant effect with CYP27B1 markers. In contrast, VDBP locus does not show interaction with the tested vitamin-D pathway genes.

The lack of association of VDBP locus variants with progression is in accordance with previous studies (Alonso et al., 1990; Cleve et al., 1988) discarding the earlier described association of VDBP variability with susceptibility to HIV-1 infection and progression to AIDS (Eales et al., 1987). In addition, our findings also evidence the lack of VDBP locus interaction with the tested vitamin-D pathway genes in the context of HIV-1 infection. Although the important role of VDBP on vitamin-D metabolism and the potential role of vitamin-D-binding protein derived factors, such as GcMAF, on immune activation, we cannot assign a relevant effect of VDBP locus variability on HIV-1 infection.

It has been proposed that vitamin-D metabolism is considerably altered during antiretroviral therapy as a result of the inhibitory effect of certain drugs on key vitamin-D pathway metabolic enzymes such as CYP27B1 (Cozzolino et al., 2003). In the present study, we have not found a significant effect of CYP27B1 locus variability on HIV-1 infection including any effect regarding treated and non-treated HIV-1 infected patients.

Among the mechanisms involved on an effective immune response against viral infections, including HIV-1, the induction of the synthesis of antimicrobial peptides and the role of T-helper mediated response should be mentioned. Remarkably, in both processes vitamin-D plays an important role with opposite effects. On one hand, the hormone promotes the synthesis of peptides such as cathelicidin and defensin-β2 that exert antimicrobial effects against to the virus entry. On the other hand, vitamin-D is known to alter both, Th1/Th2 and Th17/Treg balances, towards a Th2 and Treg response, that in the context of viral infections should be considered detrimental (Bruce et al., 2010).

Vitamin-D action is mediated by its nuclear receptor VDR. VDR gene variability has been evaluated in relation to several immune diseases. Variations in the 5′UTR VDR region exert functional effects altering binding of transcription factors to the promoter region of the gene. Thus, the 5′UTR rs4516035-G allele has been associated with lower response polarization towards Th2 induced by GATA3 (Halsall et al., 2004). According to this, the protective effect that we attribute to rs11568820-G:rs4516035-G haplotype could be explained by a lower response polarization to Th2. In addition, several studies have depicted the impact of 3′UTR variations in gene expression and mRNA stability. Haplotypes containing the 3′ UTR rs1544410-G:rs17878969-L alleles has been associated with lower levels of gene expression (Morrison et al., 1992; Torres et al., 2010) and less mRNA stability (Fang et al., 2005), which could hamper vitamin-D signalling weakening the polarization towards Th2 and Treg response. Following this, the adverse effect that we attribute to non carriers of rs1544410-G:rs17878969-L haplotype could be argued by a higher response to vitamin-D, bolstering polarization to Th2 and Treg response that, in the context ofHIV-1 infection, could be considered unfavourable.

Vitamin-D deficiency is a common feature in the general population and there is clinical evidence indicating that a vitamin-D deficiency is associated with a poor prognosis on HIV-1 infection. Whether the deficiency is a result of the infection or one of the causes of its worsening is still a controversy. The relationship of vitamin D to HIV viral load, and CD4+ T cells count has been recently evaluated by a cross-sectional study in a cohort of 112 HIV infected adults (Bearden et al., 2013). The study shows no association between vitamin D levels and CD4 cell count, whereas a U-shaped relationship between vitamin-D and viral load was observed, with the lowest and highest vitamin D levels concurrent with high viral loads. This clinical evidence is in agreement with our

association results indicating that a genetic component at the VDR locus that promotes the activation of the vitamin-D response is unfavourable in HIV-1 infection.

Serum vitamin-D has systemic effects and is responsible for the control of mineral metabolism and so-called hormone “classic effects”. However, the hormone synthesized in extra-renal tissues, such as macrophages and dendritic cells, acts locally in a paracrine/autocrine manner being responsible of local immune effects of vitamin-D. The synthesis of vitamin-D in extra-renal tissues can be increased in the focus of infection and trigger the immunosuppressive effects of the hormone. In a genetic environment that favours vitamin-D response, such as the VDR variations resulting in greater mRNA stability and enhanced expression of the gene, the immunosuppressive effect could be intensified. In an HIV-1 infected patient the exacerbation of the immunosuppressive effect caused by vitamin-D could induce a local response polarized towards Th2 and Treg, which would be unfavourable for the proper control of the infection. Notably, this response should be unrelated to the vitamin-D serum levels of the patient.Whether vitamin-D is a friend or a foe in viral infection is still unclear (Bruce et al., 2010). We acknowledge that our study have a number of potential limitations. The relatively small number of subjects in our cohort could be considered as an important limitation of the study and our results will need to be replicated in larger cohorts to confirm our findings. In addition, further analysis will be required to fully understand vitamin-D pathway role on the immune system. In the context of HIV infection, evidences for both, positive and negative effects have been debated (Bruce et al., 2010; Fibla and Caruz, 2010; Villamor, 2006), pointing the need of future studies.

Conflict of interest statement

The authors declare that they have no proprietary, financial, professional, or other personal interest of any nature that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated in this manuscript.

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Abbreviations:

SNP, single nucleotide polymorphisms

Vitamin-D, 1α,25-dihydroxyvitamin-D3

VDBP, vitamin-D binding protein

VDR, vitamin-D receptor

CYP27B1, 25-hydroxyvitamin-D3 1-α-hydroxylase

GcMAF, Macrophage Activating Factor

DCs, dendritic cells

UTR, untranslated region

AIDS, acquired immunodeficiency syndrome

HIV-1, human immunodeficiency virus Type 1

CDC, Center for Disease Control

CVC, cross-validation consistency

MDR, multifactor dimensionality reduction

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Figures

Fig. 1. Diplotype prevalence distribution by outcome according to progression criteria established in the CDC 1993. A) CYP27B1 locus diplotype prevalence. B) VDR 5′UTR locus diplotype prevalence. C) VDBP locus diplotype prevalence. D) VDR 3′UTR locus diplotype prevalence.

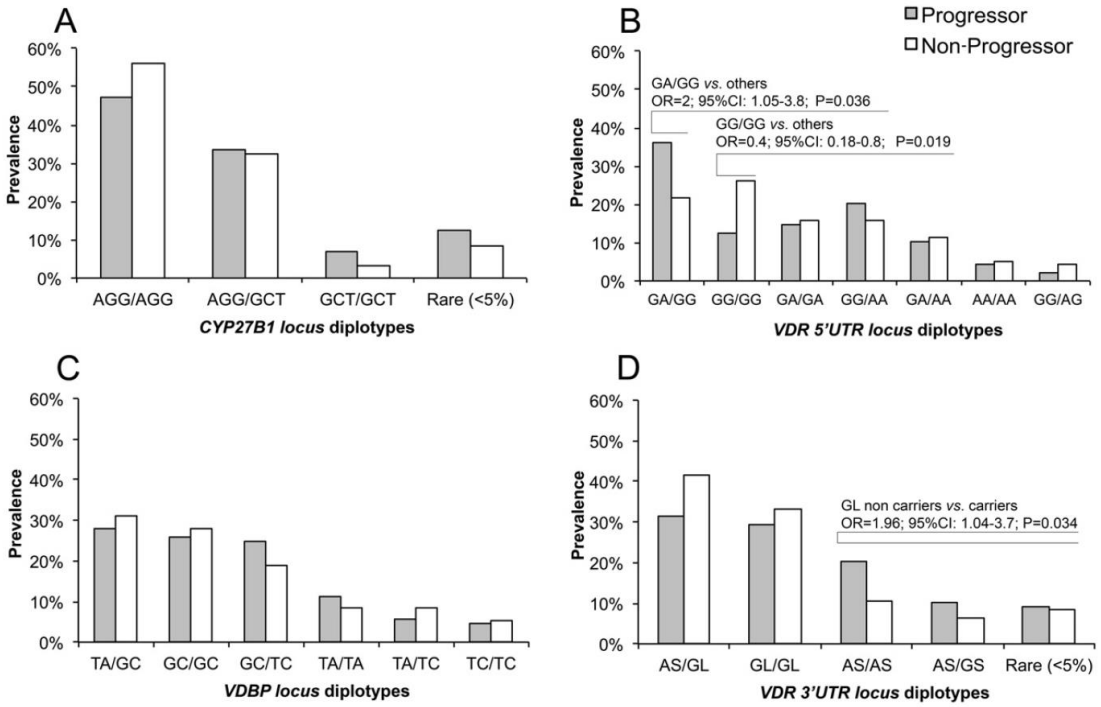

Fig. 2. Cumulative genetic score at VDR locus by outcome according to progression criteria established in the CDC 1993. A) Prevalence of progressors and non-progressors among CGS groups. B) Relative prevalence of progressors and non-progressors among CGS groups.

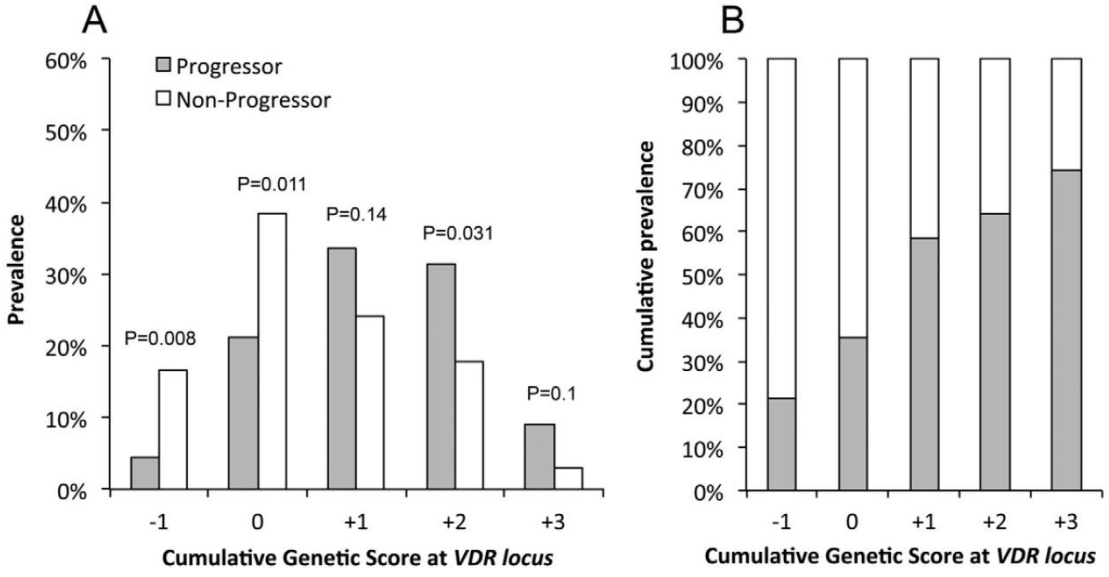

Fig. 3. Kaplan–Meier survival plot of patients reaching the outcome established by CDC 1993 during follow-up grouped by their cumulative genetic score.